

TECHNICAL SUPPORT DOCUMENT FOR CANCER POTENCY FACTORS

APPENDIX C

**Use of the Toxicity Equivalency Factor (TEF_{WHO-97} and TEF_{WHO-05}) Scheme for
Estimating Toxicity of Mixtures of Dioxin-Like Chemicals**

SRP DRAFT

EXECUTIVE SUMMARY

The widely used Toxicity Equivalence Factor (TEF) methodology for assessing the risks from exposure to dioxin and dioxin-like (DL-) compounds is based on the concept that biochemical and toxicological effects of these compounds are mediated by their binding to a receptor, Aryl Hydrocarbon Receptor (*AhR*), which controls expression of various genes by interacting with DNA. It is assumed that the effects of different compounds of this type are additive for low-level exposures. Since its initial development in 1983, the TEF methodology has continuously evolved, taking advantage of the most recent findings in toxicology studies. Although several limitations in the TEF approach have been identified, it is the best currently available method to evaluate the health risk from complex mixtures of dioxins and DL-compounds, which are commonly found both in environmental media (air, water, sediments etc.) and in biological samples (plant, animal and human tissues).

Numerous countries including the United States have adopted the WHO TEF methodology. In 2005, the WHO reevaluated human and mammalian TEFs for dioxins and DL-compounds. They utilized unweighted relative effect potency distribution ranges, expert judgment, and point estimates in combination to assign TEFs. This document updates the background and methodology for use of the TEF method for dioxins and DL-compound in the Air Toxics Hot Spots program, based on the latest WHO analysis and table of TEFs. This document also shows the difference between the latest WHO TEF and the earlier versions, and provides a summary of the history, mechanism, rationale, application, and uncertainty of TEF in risk assessment. By using the WHO TEF values, the dose response for the mixture can be predicted by calculating the sum of the potency-adjusted doses (as 2,3,7,8-TCDD or “dioxin”) of the individual compounds (The Toxic Equivalents or TEQ value). This may be used to estimate cancer risks for exposure to dioxin and DL-compounds, by multiplying the TEQ by the separately established cancer potency for 2,3,7,8-TCDD.

INTRODUCTION

It has been recognized for many years that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and a group of related chlorinated compounds (often collectively referred to as “dioxins”) are ubiquitous environmental contaminants, principally derived from combustion sources. They are extremely toxic, with a wide range of effects at low doses including carcinogenicity, immunotoxicity, reproductive and developmental toxicity. These observations were described in a report to the Scientific Review Panel (SRP) for Toxic Air Contaminants (TACs) (CDHS, 1986). On this basis the California Air Resources Board (ARB) in 1986 identified TCDD and other dibenzo-*p*-dioxins and dibenzofurans chlorinated in the 2, 3, 7, and 8 positions which contain 4 to 7 chlorine atoms as TACs for the purposes of the California TAC Program. However, the initial analysis by CDHS (1986) was based on the limited data available at that time, especially the bioassays of TCDD and hexachlorodibenzo-*p*-dioxin (HCDD).

In 1993, the California Legislature amended the California TAC Program by requiring the ARB to identify the US EPA’s 189 Hazardous Air Pollutants (HAPs) as TACs. This significantly broadened the identification of chlorinated dioxins and related compounds and added the polychlorinated biphenyls (PCBs, a specific category in the HAP list). Additionally, all the halogenated dibenzo-*p*-dioxins, dibenzofurans and biphenyls are identified as TACs under the general definition of polycyclic organic matter (POM).

The original report on chlorinated dioxins and dibenzofurans (CDHS, 1986) identified carcinogenicity as the critical effect for defining risk to public health and calculated a potency (slope factor) for TCDD of 1.3×10^5 (mg/kg-day)⁻¹. This was based on the incidence of liver tumors in male mice in a gavage study (NTP, 1982) and was calculated to be equivalent to a unit risk of 38 (μg/m³)⁻¹ for airborne exposures.

In 1996, US EPA developed three sets of PCBs cancer slope factors ranging from 0.07 to 2.0 (mg/kg-day)⁻¹ based on the 1996 GE rat study (US EPA, 1996). Warren *et al.* (2004) estimated an oral cancer slope factor of 0.27 (mg/kg-day)⁻¹ for Aroclor 1268 (Warren *et al.*, 2004).

Simon *et al.*, (2008) calculated cancer slope factor estimates for 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) that ranged from 6×10^{-2} to 6×10^{-3} (ng/kg/day)⁻¹, based on the lifetime average liver and adipose tissue concentration data from the two-year NTP study in female Sprague-Dawley (SD) rats (Simon *et al.*, 2008).

TOXIC EQUIVALENCY FACTORS

At the time of the 1980 National Toxicology Program (NTP) study (NTP, 1980), the only other chlorinated dioxins, beside TCDD, for which there were any data suitable for potency calculation were 1,2,3,6,7,8- and 1,2,3,7,8,9-HCDD, which were tested by NTP as a binary mixture in rats and mice. However, polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) and DL-PCBs (Figure 1) are the most commonly monitored dioxins in abiotic and biotic samples. These compounds occur in the environment as complex mixtures of a large number of different congeners with varying degrees and positions of chlorine substitution. These congeners are believed to have different

carcinogenic potencies (and effectiveness in causing other toxic effects typical of chlorinated dioxins and dibenzofurans). In order to calculate the potency of these complex mixtures, the TEF approach was developed to express estimates of the carcinogenic potencies of various dioxin and dibenzofuran congeners relative to that of TCDD (van den Berg *et al.*, 2000). Thus, a TEF indicates an order of magnitude estimate of the toxicity of a compound relative to TCDD, and numerous toxicological end points beside carcinogenicity are considered. Careful scientific judgment based on the examination of all available scientific data is used to derive consensus TEF values. Van den Berg *et al.* (1998) were members of the expert committee panel who evaluated the scientific information used to derive TEF_{WHO-97} values. Scientific publications selected for this purpose are included in a database based on specific criteria (van den Berg *et al.*, 1998) such as:

1. At least one PCDD, PCDF, or PCB congener and a reference compound must be included in the study.
2. Either TCDD or PCB 126 must be included as a reference compound in the same experiment or studied with the same experimental design by the same authors in another experiment.
3. The relevant end point should be affected by the congener studied as well as the reference compound.

Compounds included in the database for the TEF scheme meet criteria of inclusion described in the next section. These compounds are the 2,3,7,8-PCDDs, PCDFs, and those PCBs with established DL-activity, especially the non- and mono-*ortho* PCBs.

The US EPA (2000) report, "Exposure and Human Health, Reassessment of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) and Related Compounds", devotes a chapter to this topic. The use of TEFs is widespread in the scientific literature, but its usage as a key component of risk assessment has not been without controversy. In response to this controversy, the World Health Organization (WHO) has suggested that the Toxicity Equivalency (TEQ) scheme be reevaluated every 5 years and that TEFs and their application to risk assessment be re-analyzed to account for new scientific information (van den Berg *et al.*, 1998).

In 2005, the WHO reevaluated human and mammalian TEFs for dioxins and DL-compounds. They utilized unweighted relative effect potency distribution ranges, expert judgment, and point estimates in combination to assign TEFs (van den Berg *et al.*, 2006).

The NTP (2006) carried out a series of studies in which rodents were exposed to either a single dioxin-like compound or mixtures of them for up to two years. The investigators assessed the toxicity and carcinogenicity of the DL compounds relative to TCDD alone. The NTP report states "Analysis of data from one group of completed studies confirms the assumption that the effects of the dioxin-like compounds in mixtures are additive. The number of cancer cases in the rats exposed to the mixture could be predicted accurately by adding the concentration of each compound, adjusted for its potency relative to TCDD using TEFs."

HISTORY OF TEF DEVELOPMENT

In 1983, based on a review of the scientific information available at the time, the Ontario Ministry of Environment (OME) set the basis for the TEF methodology by introducing the concept that PCDDs and PCDFs:

1. Are structurally similar compounds that share a common cellular mechanism of action - activation of the aryl hydrocarbon receptor (*AhR*), and
2. Produce similar biological and toxicological responses.

They also proposed that the toxic response of these compounds could be added up using a “toxic equivalence” scheme with 2,3,7,8-TCDD as a reference compound to assess human risk (OME, 1984).

In the original health risk assessment for PCDDs and PCDFs conducted under the California TAC program (CDHS 1986), the Department of Health Services utilized two cancer bioassays conducted by the NTP to estimate TEFs for the 2,3,7,8-chlorinated congeners (Table 1). These California TEFs were eventually replaced when the International Toxicity Equivalency Factor (I-TEF) scheme (described below) was adopted by OEHHA, following review by the SRP in 1999.

In an analogous fashion to OME’s and California’s approaches, US EPA developed its own TEF methodology to characterize toxicity of complex mixture of PCDDs/PCDFs from waste incinerators. Rather than dividing PCDDs and PCDFs congeners into their respective homologue groups and then assigning a TEF number to each group, as proposed by OME, US EPA recommended that each congener receive a TEF number. The concentration of each monitored PCDD and PCDF congener could then be multiplied by its respective TEF value and all the products summed to give 2,3,7,8-TCDD equivalent concentration (TEQ) (US EPA, 1987). This approach is mathematically described as:

$$\text{Total Toxicity Equivalence (TEQ)} = \sum_{n=1}^k (C_n * TEF_n)$$

where TEF_n = toxic equivalency factor of individual congener, C_n = concentration of the individual congener in the complex mixture, and k = total number of congeners.

In this approach, TEFs are determined by inspecting the available congener-specific data and by assigning an “order of magnitude” estimate of relative toxicity when compared to 2,3,7,8-TCDD. Scientific data considered in this scheme included *in vitro* binding to the *AhR* and *in vitro* and *in vivo* toxicity studies.

Subsequently, the North Atlantic Treaty Organization Committee on the Challenges of Modern Society, after conducting a 3-year study (NATO/CCMS, 1988), proposed from international consensus an I-TEF scheme. The committee recognized that the data reviewed in its study strongly support the role of the *AhR* as a mediator in the biological and toxic response of 2,3,7,8-TCDD. Improvement from previous efforts in the determination of TEFs

included selection of TEF values based more on *in vivo* toxicity testing, assigning TEF values to octachlorodibenzo-*p*-dioxin and octachlorodibenzofuran and removing any TEF values for non-2,3,7,8-substituted congeners. US EPA officially adopted the revised I-TEF in 1989 as the preferred method of calculating risks from exposure to DL-compounds, but with the recommendation that this risk assessment approach remains interim and that continued revisions should be made. Canada, Germany, Italy, the Netherlands, Sweden, the United Kingdom, and the United States formally adopted the use of I-TEF model for risk assessment and risk management purposes (Yrjänheiki, 1992). This so-called “I-TEF” scheme was also used in the California air toxics programs. It was recommended for use in the Hot Spots program (AB 2588) by OEHHA (1999), and adopted after review by the SRP.

As TEFs for PCDDs and PCDFs were developed, considerable efforts went into the study of quantitative structure activity relationships (QSAR) for PCBs. PCB congeners substituted in the *para* and at least 2 of the *meta* positions but not at any of the *ortho* positions can adopt structural conformations most resembling that of 2,3,7,8-TCDD, therefore have the greatest potency and exert their toxicity through the *AhR* pathway. These coplanar PCB congeners are structurally similar to 2,3,7,8-TCDD and therefore are termed DL-PCBs. Introduction of one chlorine in the *ortho* position results in a decrease in toxic potency and PCBs with more than one chlorine in the *ortho* positions lack some effects exerted by non- and mono-*ortho* PCBs. These PCB congeners show a different spectrum of toxic effects (Safe, 1994).

In 1991, US EPA considered using the TEF methodology for PCBs. They noted that only a small subset of the 209 PCB congeners elicits DL-activity and meet the criteria for inclusion in the TEF methodology.

In an attempt to harmonize TEF schemes for DL-compounds, the World Health Organization - European Center for Environmental Health (WHO-ECEH) and the International Program on Chemical Safety (IPCS) generated a database consisting of almost 1,200 peer-reviewed publications, representing all the available toxicological data for PCBs up to the end of 1993. From a selected number of these publications and based on four inclusion criteria, the WHO-ECEH and the IPCS proposed TEF values for 13 DL-PCBs (Ahlborg *et al.*, 1994). The inclusion criteria are:

1. The compound should show structural similarity to PCDDs and PCDFs.
2. It should bind to the *AhR*.
3. It should induce dioxin-specific biochemical and toxic responses.
4. It should be persistent and accumulate in the food chain.

In addition, the first WHO PCB TEF consultation (Ahlborg *et al.*, 1994) recommended expanding the current database to include all relevant information on PCDDs, PCDFs and other DL-compounds that satisfied the four inclusion criteria.

Some terminologies and definitions applicable to TEFs were reviewed prior to the second WHO-ECEH consultation (van Leeuwen, 1997). The term TEF, used in the past to describe any experimental end point to be compared with TCDD, was reconsidered since not all end points are “toxic” end points. For example, end points such as binding to the *AhR* and

induction of ethoxyresorufin-O-deethylase (EROD) are mostly considered biological/biochemical responses. Therefore, experimental end points, for which numerical values are compared to the response to TCDD, should be termed “Relative Potency” values (REPs). These REPs could be the result of a single laboratory experiment looking at a single end point. REPs are derived from the available data either used as reported in each publication, or calculated by comparing dose-response curves or ratios of medium effective doses (ED_{50}), median lethal dose (LD_{50}), and median effective concentration (EC_{50}), etc. A chemical’s TEF is then derived from all available REPs examined for that compound. Thus, the term TEF is restricted to describe an overall estimate of the order-of-magnitude of the toxicity of a compound relative to the toxicity of TCDD. This estimate is derived by consensus, using careful scientific judgment of all available data (van Leeuwen, 1997; van den Berg *et al.*, 1998). The derivation of TEF consensus values using *AhR*-specific end points gives more weight to toxic responses than to biochemical (e.g., enzyme induction) responses and it puts more weight on *in vivo* data than on *in vitro* results. In addition, the weighting order of contributing *in vivo* data was: chronic > subchronic > subacute > acute.

In 1997, the WHO-ECEH proposed amendments to the previous NATO/WHO I-TEF scheme (NATO/CCMS, 1989). For revision of the existing mammalian TEFs, the WHO-ECEH committee agreed that if the available information was considered insufficient to warrant a change, the existing value would remain. The suggested WHO-97 TEFs for humans and mammals are presented in Table 1.

In 2005 the WHO expert panel (van den Berg *et al.*, 2006) suggested that the “ideal” REP study design should follow general guidelines for a future ideal dose-response study used to determine an *in vivo* REP:

1. A full dose-response curve for both the congener and for 2,3,7,8-TCDD should be determined.
2. The congener and 2,3,7,8-TCDD should be administered by the same route to animals of the same species, strain, sex, and age, and the animals should be housed, fed the same diet, and maintained under the same conditions in the same laboratory.
3. Ideally, the absolute maximal response (efficacy) should be similar for both the congener and for 2,3,7,8-TCDD and their dose-response curves should be parallel, but in practice, this is often not observed for various reasons.
4. If the above dose-response criteria are met, the REP should be calculated by dividing the effective dose 50% (ED_{50}) of 2,3,7,8-TCDD by the ED_{50} of the congener.
5. If full dose-response relationships are not attained and determination of ED_{50} s is not possible, lowest observed effect doses or concentrations or benchmark doses could be used to determine the REP. However, such an REP has more uncertainty than if ED_{50} s were used.

For *in vitro* studies, which are from an experimental design point of view usually easier to accommodate than *in vivo* studies, the following experimental design is suggested to determine an REP (van den Berg *et al.*, 2006):

1. A vehicle group and at least four graded concentrations of a congener and four graded concentrations of 2,3,7,8-TCDD should be selected.
2. For congener and 2,3,7,8-TCDD treatment groups, three of these concentrations should elicit a response that falls between the EC₂₀ and EC₈₀ for the congener and for 2,3,7,8-TCDD.
3. At least one concentration should elicit a maximal response (EC₁₀₀), and the concentration-response curves should be parallel.
4. The REP should be based on the EC₅₀ of 2,3,7,8-TCDD and the EC₅₀ of the congener.

Table 1 represents some of the various iterations in the TEF scheme development, which have been referenced in earlier California risk assessment guidance. For each of these iterations, it was noted that although TEF methodology is subject to several criticisms, it is still the best available approach to assess health risks from mixtures of DL-chemicals. The WHO-ECEH recommended that the TEF methodology be reevaluated every five years in order to account for new scientific findings in this area. In 2002, WHO designated the Institute for Risk Assessment Sciences (IRAS) in Utrecht, Netherlands, as a Collaborating Centre, and their work plan includes the updating of the database on comparative toxicity of PCDDs and PCDFs. (Dr. Maged Younes, Department of Protection of the Human Environment, WHO, 2002, personal communication). In the most recent TEFs in 2005, WHO reevaluated human and mammalian TEFs for dioxins and DL-compounds and made some changes (Table 1) (van den Berg *et al.*, 2006). We propose to adopt those new WHO 2005 TEF values for use in risk assessment under the Air Toxics Hot Spots program.

PHYSIOLOGICAL BASIS OF THE TEF METHODOLOGY: THE *Ah* RECEPTOR

Mechanism of Dioxin Toxicity

Many PCDDs, PCDFs, coplanar PCBs, and other structurally related polyhalogenated aromatic hydrocarbons are believed to share a common mechanism of action intimately related to similarities in their structural configuration. Most of what is known regarding the mechanism of action of these compounds is derived from research in three areas: structure-activity relationships for receptor binding and induction of a variety of biochemical and toxicological responses; genetic studies using inbred mouse strains; and studies at the molecular level that have elucidated key events in the actions of the receptor (Pohl *et al.*, 2000).

Most mechanistic studies to date support the assumption that binding to the *Ah*R is a key first step prior to DL-compounds eliciting a toxic and biochemical response. There are numerous reviews available on this subject (Birnbaum, 1994a, b; Okey *et al.*, 1994; Pohl *et al.*, 2000; Poland and Knutson, 1987; Safe, 1990). Perhaps the strongest support of the role of the *Ah*R in mediating the toxicity of 2,3,7,8 -TCDD and related compounds arises from genetic data.

Responsiveness of certain mouse strains to 2,3,7,8-dibenzo-*p*-dioxin toxicity appears to segregate with the presence of the *AhR* locus (Poland and Glover, 1980). For instance, the C57BL/6J mouse has an *AhR* protein with a relatively high binding affinity for inducers of AHH such as 3-methylcholanthrene, β -naphthoflavone, 2,3,7,8-TCDD, and other isostereomers of 2,3,7,8-TCDD, and is sensitive to the toxic effects of these chemicals. In contrast, the DBA/2 mouse has an *AhR* protein that has a lower ligand affinity (Okey *et al.*, 1989), and is much less sensitive to the toxic effects of these compounds. Fernandez-Salguero *et al.* (1996) also demonstrated the role of the *AhR* protein in mediating the toxicity of 2,3,7,8-TCDD and other DL-compounds by using an *AhR*-deficient mouse strain. Thus, the hypothesis that TCDD and related compounds toxicity are initiated by binding to the *AhR* is the general basis for the TEF scheme (Safe, 1990; Okey *et al.*, 1994; Birnbaum, 1994a, b; Hankinson, 1995). The events following *AhR*-ligand binding that lead to the toxic response are not fully established.

The *AhR* is a cytosolic protein present across species in target tissues and organs. This protein is a member of the basic Helix-Loop-Helix-Per-Arnt-Sim (bHLH-PAS) family of transcription factors. When bound to a ligand, the *AhR*-ligand complex associates with another bHLH-PAS protein, the *AhR* nuclear translocator (ARNT), in the nucleus of cells (Holmes and Pollenz, 1997). The heterodimeric DNA-binding protein complex, *AhR*-ARNT, binds to the xenobiotic response elements (also termed dioxin response elements or DREs) of the DNA located near the 5' regulatory region of genes such as the CYP1A1 gene (Fisher *et al.*, 1989). DREs are dioxin-responsive regulatory DNA domains, which have the properties of a transcriptional enhancer (Fisher *et al.*, 1990; Neuhold *et al.*, 1986). They require both receptor protein and ARNT protein for enhancer function. The binding to the DRE of the heterodimeric DNA-binding protein complex modulates the transcription of various genes like the CYP1A1 gene (Kawajiri *et al.*, 1995). In addition to the enhancer, the DNA upstream of the 1A1 gene has a second control element (a transcriptional promoter), which ensures that transcription is initiated at the correct site. Neither enhancer nor promoter functions in the absence of the other (Jones and Whitlock, 1990).

2,3,7,8-TCDD and related compounds, after binding to the *AhR*, have been shown to alter the transcription and/or translation of a number of genes. These include several oncogenes and genes encoding growth factors, receptors, hormones and drug metabolizing enzymes (Birnbaum, 1994a, b). Also affected are certain enzymes and proteins (e.g., kinases) involved in various signal transduction processes, as well as cell cycle control (Birnbaum, 1994a, b; Weib *et al.*, 1996). *AhR*-mediated gene expression is also involved in several critical life processes (e.g., cell type-specific differentiation, cell division, apoptosis) via signal transduction mechanisms (Micka *et al.*, 1997).

The activation of genes encoding for drug-metabolizing enzymes is important in both the activation (by metabolism of substrates to genotoxic reactive intermediates or ultimate carcinogens) and the detoxification of toxic or carcinogenic drugs and other environmental pollutants. The elicited induction of certain drug metabolizing enzymes such as the cytochrome P450 1 subfamily (CYP1A1, CYP1A2 and CYP1B1) is one of the most sensitive responses observed in a variety of different animal species, including humans. However, there is still a gap between knowledge of these changes and the degree to which they are

related to the biological and toxicological end points elicited by 2,3,7,8-TCDD and related compounds (Pohl *et al.*, 2000).

There is some evidence suggesting that other *AhR*-mediated pathways, not dependent on the interaction of the *AhR* with nuclear elements, may exist for the alteration of gene expression. Matsumura (1994) suggested that the interaction of 2,3,7,8-TCDD with the *AhR* may initiate a phosphorylation/dephosphorylation cascade, which would subsequently activate other transcription factors. An increase in protein kinase activity was observed within 1-10 min following the addition of 2,3,7,8-TCDD to nuclear-free preparations of guinea pig adipose tissue (Enan and Matsumura, 1996). As discussed by Pohl *et al.* (2000), 2,3,7,8-TCDD may modulate signal transduction processes and gene expression by at least two pathways: through the direct interaction of the *AhR* and its heterodimer partners with gene regulatory elements, and from the initiation of a phosphorylation/dephosphorylation cascade and the subsequent modulated activity of other nuclear transcription factors. The prominent pathway may differ for acute versus chronic responses to the DL-compounds and for particular developmental periods.

Ligand binding also results in rapid depletion of *AhR* in both cell culture and animal studies (Pollenz *et al.*, 1998; Roman *et al.*, 1998). These findings lead to the hypothesis that a decrease in *AhR* protein may be important in the regulation of *AhR*-mediated signaling (Pollenz *et al.*, 1998). Thus, regulation of the *AhR* could have important impacts on the toxicological outcome of exposure to dioxin and DL-chemicals. In developing rats, *AhR* protein levels in ventral and dorsolateral prostate decrease with age, declining approximately 70% between postnatal day (PND) 1 and 21. Similar decreasing trend was found for ARNT protein levels in dorsolateral but not ventral prostate (Sommer *et al.*, 1999). This decrease was associated with a decrease in *AhR* and ARNT mRNA. TCDD (0.2, 1, 5, or 25 µg/kg po, 24 h) treatment of adult male rats decreased *AhR*, but not ARNT, protein in ventral and dorsolateral prostate, vas deferens, and epididymis (Sommer *et al.*, 1999). The study also showed that perinatal TCDD exposure (1.0 µg/kg po) decreases prostatic *AhR* protein levels on PND 7. Pretreatment of rat pups for 24 h with TCDD (5 µg/kg ip) down-regulated prostatic *AhR* protein on postnatal day 7, but not on PND 1. The authors concluded that prostatic *AhR* and ARNT protein and mRNA levels are regulated with age, whereas only *AhR* protein concentration is altered by TCDD exposure.

Polymorphism of the *Ah* Receptor

There is great variability in the response of individuals following exposure to dioxins. In mice, a polymorphism based on a single nucleotide difference in the ligand binding domain of the *AhR* is sufficient to reduce the affinity for ligands by more than 10-fold in non-responsive strains (Fernandez-Salguero *et al.*, 1996; Wong *et al.*, 1997). In humans, Needham *et al.* (1998) reported, in a follow-up study of the Seveso incident, that the mean serum lipid TCDD concentration of children with chloracne was 18,700 ppt with a range of 1,680 to 56,000 ppt. Yet, other individuals with as high or higher serum dioxin levels did not develop chloracne. The individual variability in sensitivity to TCDD was proposed to be due to polymorphism in the *AhR*. In addition, Needham *et al.* (1998) reported a longer half-life in serum TCDD for women and a biphasic half-life with an initial rapid phase in children, when compared to adult men (average half-life for TCDD = 7.8 years). The average half-life (geometric means) for

total PCBs in occupationally exposed women is 19.8 years that is significantly longer than that in occupationally exposed men (9.0 years) (Seegal et al., 2010). A similar gender effect is apparent for EROD activity and CYP1A1 protein levels (by immunoblotting) which are lower in women compared to men (means = 4.50 vs. 9.01 pmol/min/mg protein and 10,520 vs. 17,246 U, respectively) (Smart and Daly, 2000), suggesting gender-specific polymorphism for the *AhR*. The gender difference for the *AhR* was also demonstrated in rodents and in nonhuman primates. Furthermore, *in vitro* glucose uptake by adipose tissue was decreased in male guinea pigs treated with TCDD, but not in females. A similar pattern of response was observed in macaques (Enan *et al.*, 1996). TCDD induced lipid peroxidation in the adipose tissues was found in male but not female guinea pigs (Enan *et al.*, 1996). TCDD binding affinity studies in adipose explant tissues showed that tissues from male guinea pigs and monkeys had a higher binding capacity for TCDD than that from female tissues.

In addition to gender difference of the *AhR* polymorphism, *AhR* activities are also enzyme and tissue-specific. A ninety-day study of PCB congeners in mice showed that *Ah*-receptor activities are tissue and enzyme-specific with wide variability. For example, the liver acetanilide 4-hydroxylase (ACOH)/EROD ratios of relative potency (REP) for PCB 77 and PCB 118 are 10- and 150, respectively, whereas the ratios of liver/lung EROD induction from PCB 77 and PCB 118 are 0.17 and 4, respectively (Birnbaum and DeVito, 1995). *In vitro* studies of liver enzyme induction show that for *Ah*-receptor binding biomarkers, calculated as half maximal effective concentration (EC₅₀) values, there is still large variation among the congeners. The ratios of TEF_{RBA} (receptor binding affinity) / TEF_{EROD} for PCB 77 and PCB 118 are 99- and 469-fold respectively (Giesy and Kannan, 1998).

As in experimental animals, human populations exhibit a greater than 20-fold range in the CYP1A1 inducibility/*AhR* affinity phenotype (Micka *et al.*, 1997). Determination of binding affinity toward TCDD in 86 human placenta samples showed a greater than 20-fold range in binding affinity. This range encompasses binding affinities similar to those observed in sensitive and resistant mice (Okey *et al.*, 1997).

Incubating lymphocytes from 30 volunteers (20 women and 10 men, age 25 – 37) with 3-methylcholanthrene for 96-h revealed human inter-individual variation of CYP1A1 and *AhR* gene polymorphisms, in which the inducibility of CYP1A1 activity is in the order of 103-fold (Smart and Daly, 2000). However, this study did not show any association between induced CYP1A1 activity and the presence of the novel alleles identified by single-strand conformational polymorphism (SSCP) analysis. Four genetic polymorphisms within the CYP1A1 gene have been described and those alleles were termed: CYP1A1*2A, CYP1A1*2B, CYP1A1*3 and CYP1A1*4. The polymorphism coded by the allele CYP1A1*2A has been associated with increased lung cancer in a Japanese population, whereas a lower frequency for this polymorphism is found in the Caucasian population. The allele CYP1A1*3 is specific to the African American population, and was not clearly associated with lung cancer susceptibility. Therefore, various races may have different dominant CYP1A1 polymorphism. As for the recently identified polymorphism CYP1A1*4, its function has not yet been elucidated. The SSCP methodology used in this study has been estimated to detect at least 80% of point mutations.

AhR polymorphism could alter CYP1A1 inducibility. Screening for the 11 exons of the *AhR* gene by SSCP analysis confirmed the existence of the previously described G1721A (guanine at position 1721 replaced by adenine) polymorphism in a Caucasian population and found a novel G1768A polymorphism (Smart and Daly, 2000). Individuals with at least one copy of the G1721A *AhR* variant allele showed a significantly higher inducibility of CYP1A1 activity compared with individuals without the occurrence of the polymorphism ($p = 0.0001$) (Smart and Daly, 2000). Similar findings were obtained for induced CYP1A1 protein levels as determined by immunoblotting. It appears that the genotype for the *AhR* G1721A polymorphism shows a better correlation with induced CYP1A1 levels than several other factors, including the genotype for several previously described CYP1A1 alleles. In the same line, Wong *et al.* (2001) reported two polymorphisms in *AhR* that show apparent linkage disequilibrium with the codon 554 polymorphism: 1) a previously described polymorphism, V570I; 2) a novel human *AhR* polymorphism, P571S. The authors noted that neither of these variants showed abnormal ligand binding or DNA binding activities in an *in vitro* assay. However, the combined Ile (570) + Lys(554) variant *AhR* form failed to support induction of CYP1A1 in cells treated with TCDD (Wong *et al.*, 2001). This rare combination of variant genotypes appears to be restricted to individuals of African descent. The authors postulated that this genotype might result in reduced susceptibility to the carcinogenic effects of polycyclic aromatic hydrocarbons (PAHs).

Therefore, gender and *AhR* polymorphism could be determinants of the level of induced CYP1A1 activity, and inter-individual variation in levels of induced CYP1A1 activity could be associated more with regulatory factors than polymorphism in the CYP1A1 gene (Smart and Daly, 2000). Good correlations have been found between structural polymorphisms in the gene and functional variants in various genetic strains of mice. In humans, however, work on polymorphisms and their possible role in gene function and cancer susceptibility is at a relatively early stage (Garte and Sogawa, 1999).

Ligands for the *Ah* Receptor

AhR ligands, including 2,3,7,8-TCDD (dioxin), are the most toxic members of the polyhalogenated aryl hydrocarbon (PHAH) family. The binding of PHAHs to the *AhR* protein is an essential step in eliciting DL-effects. Several other PHAHs such as PCDDs, PCDFs, and non-*ortho* PCBs also bind to the *AhR* and induce toxic responses similar to those observed with TCDD. Those chemicals are industrial compounds or by-products that have been widely identified as environmental contaminants and detected in fish, wildlife, and humans. Moreover, for these PHAHs there is a rank order correlation between their structure-*AhR* binding affinities and their structure-toxicity relationships. This supports a role for the *AhR* in mediating these responses (Safe, 1998).

One of the criteria for the inclusion of anthropogenic chemicals in the TEF methodology is their persistence and bioaccumulation in wildlife and humans. There are other anthropogenic and naturally occurring chemicals capable of binding to the *AhR*. However, these chemicals are not included in the TEF scheme since they are either ligands with weak affinity for the *AhR*, elicit toxic responses that are mostly mediated through a pathway other than that mediated by *AhR*, or have a short half-life. Anthropogenic chemicals with affinity for the *AhR* include industrial chemicals (polyhalogenated biphenyls, halogenated naphthalenes,

chlorinated paraffins, etc.), pesticides (hexachlorobenzene), and contaminants (polyhalogenated dioxins and furans) associated with various manufacturing production, combustion, and waste disposal processes (US EPA, 2000). In addition, unsubstituted PAHs can also bind with moderate to high affinity to the *AhR* (Poland and Knutson, 1987; Nebert, 1989; Chaloupka *et al.*, 1993).

Chemicals such as hexachlorobenzene are only weakly dioxin-like, and have significant toxicological effects that are not mediated by the *AhR*. PAHs are not included either in the TEF scheme because of their short half-lives and relatively weak *AhR* activity. Brominated dioxins, benzofurans, biphenyls, and naphthalene also induce DL-effects in experimental animals (Miller and Birnbaum, 1986; Birnbaum *et al.*, 1991; Hornung *et al.*, 1996; DeVito *et al.*, 1997; Weber and Greim, 1997). The potency of brominated compounds in comparison to their chlorinated homologue depends on the specific congener (Birnbaum *et al.*, 1991; DeVito *et al.*, 1997). In general, exposure data for these chemicals are limited and exposure of the general population is unknown. However, there are currently concerns regarding the steadily increasing level of polybrominated diphenyl ethers (PBDEs) in the environment, wildlife, and human tissue (de Wit, 2002; Hardy, 2002; Darnerud *et al.*, 2001; She *et al.*, 2002; Vonderheide *et al.*, 2008). Thus, future TEF evaluation should investigate these chemicals. The next TEF evaluation by the WHO might consider these brominated chemicals if there are sufficient data to justify their inclusion in the TEF methodology.

Zhang *et al.* (2008) investigated structure-dependent differences in activation of the *AhR* by a series of halogenated aromatic hydrocarbons. TCDD, 1,2,3,7,8-pentachlorodibenzo-pdioxin (PeCDD), 2,3,7,8-tetrachlorodibenzofuran (TCDF), 2,3,4,7,8-PeCDF, and PCB126 induced CYP1A1-dependent activities in HEK293 human embryonic kidney, Panc1 pancreatic cancer, and Hepa1c1c7 mouse hepatoma cell lines. They found a structure-dependent difference in the efficacy of TCDF and PCB126 in HEK293 and Panc1 cells; induced CYP1A1mRNA levels were lower than observed for the other congeners. Their results of the mammalian two-hybrid studies demonstrated that activation of pGAL4-luc in cells transfected with VP-*AhR* and GAL4-coactivator chimeras is dependent on the structure of the HAH congener, cell context, and coactivator, suggesting that the prototypical HAH congeners used in their study exhibit selective *AhR* modulator activity (Zhang *et al.*, 2008).

In contrast to anthropogenic ligands for the *AhR*, naturally occurring *AhR* ligands have short half-lives, but nevertheless have frequently been cited in criticism of the TEF methodology. Naturally occurring *AhR*-ligands include: indole derivatives (indole-3-carbinol (I-3-C), 3,3'-diindolylmethane (DIM), indolocarbazoles (ICZs) etc.), heterocyclic aromatic amines (HAAs), and oxidized essential amino acids.

Indole chemicals such as I-3-C and DIM are present in a variety of cruciferous vegetables. These two major secondary metabolites are capable of inducing phase I and II metabolic enzymes (CYP1A-dependent glutathione and glucuronyl transferases, oxidoreductases) in experimental animals (Bradfield and Bjeldanes, 1984; 1987), human cell lines (Bjeldanes *et al.*, 1991; Kleman *et al.*, 1994; Degner *et al.*, 2009), and humans (Michnovicz and Bradlow, 1990; 1991). Gene expression profiling study in Caco-2 human colon cells by using microarray analysis found that similar effects for over 20 genes induced by natural *Ah* receptor agonists, TCDD and B[a]P at the transcriptome level in a human intestinal cell line in

vitro (de Waard et al., 2008). However, these compounds have a low binding affinity for the *AhR* (Gillner et al., 1985). In contrast, indolo[3,2b]carbazole exhibits high binding affinity for the rodent *AhR* (approximately equipotent to 2,3,7,8-tetrachlorodibenzofuran) and can induce CYP1A1 activity in cultured cells (Bjeldanes et al., 1991; Gillner et al., 1993; Chen et al., 1995). The ICZ family, of which indolo[3,2b]carbazole is a member, are byproducts of DIM - acid condensation reactions, or formed by bacterial metabolism of the common dietary amino acid tryptophan.

Amakura et al. (2003) reported on ninety vegetable constituents including flavonoids, tannins, saponins, terpenes, etc., that were assayed *in vitro*. Among them, flavones, flavonols, anthraquinones, piperine, coumestrol, brevifolincarboxylic acid, and resveratrol showed marked inhibitory effects on *AhR*-based bioassay activation by TCDD, and their effects were dose dependent. Curcumin, carnosol, and capsaicin also inhibited the activation of *AhR* in this assay, although to a lesser degree. These results suggest that several vegetable constituents might play a role in protection against dioxin toxicity (Amakura et al. 2003). Jeuken et al. (2003) also suggested some food extracts contained *AhR* antagonists whose effectiveness was overcome by dilution (Jeuken et al. 2003). Zhang et al. (2003) suggested that dietary phytochemicals exhibit substantial cell context-dependent *AhR* agonist as well as antagonist activities (Zhang et al. 2003).

Connor et al., (2008) reported the induction equivalent (IEQ) or IEQ concentration in human blood samples from 10 volunteers under different dietary regimens. They concluded that a substantial portion of the IEQ activity occurred as a result of the increased intake of natural *AHR* agonists (NAHRAs) present in many fruits, vegetables, and herbs (Connor et al., 2008). As they mentioned, they measured an induction equivalent (IEQ) in human blood samples from 10 volunteers under different dietary regimens. However, the study is not representative of the general population since the total sample size is 10 individuals, each with different dietary regimens. Large intraspecies difference with many potential confounding factors may exist.

Other dietary *AhR* ligands appear to be formed in cooked meat. Experimental animals and humans fed cooked meat exhibited CYP1A2 induction (Degawa et al., 1989). The CYP1A2 induction was associated with the formation of a number of heterocyclic aromatic amines (HAAs) in human volunteers (Sinha et al., 1994). Oxidized essential amino acids, such as UV-oxidized tryptophan, were also shown to induce CYP1A1 activity in mouse hepatoma cells through an *AhR*-dependent mechanism and induced hepatic and pulmonary CYP1A1 activity in rats exposed *in vivo* (Sindhu et al., 1996).

The discovery of these naturally occurring *AhR* ligands gave rise to vigorous criticism of the TEF methodology. It was proposed that calculation of the TEQ for DL-contaminants could be skewed by the (dietary) intake of relatively high level of naturally occurring *AhR* agonists (Safe, 1997) and thus lessen the suitability of the TEQ calculation. This criticism was, however, rejected in the US EPA's assessment of dioxin health effects (US EPA, 2000) and in the WHO 2005 TEF (van den Berg *et al.*, 2006). Although it was proposed that more than 90% of the TEQ is derived from natural or dietary compounds (Safe, 1995), these naturally occurring *AhR* ligands have short half-lives and generally do not bioaccumulate. In contrast, the PCDDs, PCDFs and PCBs included in the TEF methodology clearly bioaccumulate and

have long biological half-lives, typically in years. Therefore, if contributions to the total TEQ are estimated on steady-state body burdens of these chemicals instead of daily intake, then TCDD and other PCDDs/PCDFs and PCBs contribute more than 90% of the total TEQ compared to the “natural” ligands (DeVito and Birnbaum, 1996; US EPA, 2000). This difference was further characterized by comparing indolo[3,2b]carbazole potency to TCDD. After 4 hours of exposure of Hepa-1 cells to these chemicals, the relative potency of indolo[3,2b]carbazole was 0.1 that of TCDD (Chen et al., 1995). However, after 24 hours of exposure to the same compounds, the relative potency of indolo[3,2b]carbazole compared to TCDD was 0.0001 (Chen et al., 1995). Moreover, it has been argued that the characteristic DL-toxicity is only manifested by highly persistent chemicals which cause stimulation of the *Ah* pathway over long periods of time; short-term stimulation may result only in the induction of the cytochrome P450 and Phase II enzymes which are effective in clearing most *AhR*-binding chemicals from the body. OEHHHA agrees with the US EPA view.

These results illustrate the importance of considering pharmacokinetic factors in the inclusion of compounds in the TEF methodology. Although some chemicals, including chemicals that occur naturally, bind to the *AhR* and some may elicit DL-activity, it is clearly not sufficient to be considered in the TEQ calculation. Other toxicological factors, such as biological half-life, exposure and toxicity data *in vivo* should be considered.

WHO experts reported “the majority of toxicity studies demonstrated that these naturally occurring *AhR* agonists fail to produce *AhR*-dependent toxicity (Leibelt et al., 2003; Pohjanvirta et al., 2002), although some developmental DL-effects have been reported for indole-3-carbinol (I3C) (Wilker et al., 1996). In addition, naturally occurring *AhR* ligands, such as I3C and diindolymethane, have been reported to inhibit 2,3,7,8-TCDD–dependent *in vivo* induction of CYP1A1 and immunotoxicity (Chen et al., 1995, 1996). The ability of some non-dioxin–like PCBs and PCDFs to inhibit 2,3,7,8-TCDD–induced CYP1A1 activity and immunotoxicity in C57BL/6J mice has also been reported (Bannister and Safe, 1987; Biegel et al., 1989; Chen and Bunce, 2004; Crofton et al., 2005; Davis and Safe, 1988; Loeffler and Peterson, 1999; Morrissey et al., 1992; Smialowicz et al., 1997), whereas other studies have shown synergistic effects on dioxin toxicity of non-dioxin–like compounds, e.g., thyroid hormones, porphyrins, reproductive toxicity, and immunotoxicity (Bannister and Safe, 1987; Birnbaum et al., 1986; Crofton et al., 2005; Loeffler and Peterson, 1999; van Birgelen et al., 1996b). The above studies provide evidence that non-dioxin–like compounds that are weak *AhR* agonists can modulate the overall toxic potency of 2,3,7,8-TCDD and related compounds. If occurring under natural background situations, these interactions might impact the magnitude and overall toxic effects produced by a defined amount of TEQ (i.e., from intake or present in the body) but not impact the determination of individual REP or TEF values for DL-chemicals. The expert panel recognized that there are studies providing evidence that non-dioxin–like *AhR* agonists and antagonists are able to increase or decrease the toxicity of 2,3,7,8-TCDD and related compounds. Accordingly, their possible effect on the overall accuracy of the estimated magnitude of the TEQ needs to be investigated further, but it does not impact the experimental determination of individual REPs or TEFs. ” (van den Berg, et al., 2006).

BASIS OF TEF AND TEQ CALCULATION: THE ASSUMPTION OF ADDITIVITY

The TEF/TEQ methodology is based on the scientific assumption that the *Ah*R mediates the biochemical and toxicological actions of DL-chemicals. Another essential assumption in the development of the TEF methodology is the one of additive interactions. Although there are numerous scientific reports on the synergistic or antagonistic interaction of mixtures of DL- and/or non-DL-chemicals with TCDD, reports on the additive effects of DL-chemicals predominate. Several published studies aimed to validate the concept of the TEF methodology as a tool to predict the risk of exposure to DL-chemical mixture.

Groups of 20 male and 20 female rats were gavaged with five doses of a mixture of TCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, and 1,2,3,4,6,7,8-HpCDD divided into four daily loading doses and six biweekly maintenance doses (Viluksela *et al.*, 1998a). Positive control groups were treated with PeCDD and HxCDD. The animals were dosed for 13 weeks and then divided into two groups; half of the rats were killed and the rest were provided with a 13-week off-dose period. Viluksela *et al.* (1998a) reported a dose-dependent increase in liver EROD activity, an enzyme associated with CYP1A1 induction, and a dose-dependent decrease in liver phosphoenolpyruvate carboxykinase in rats dosed with the mixture. There was also a dose-dependent decrease of serum thyroxine (T₄) in the mixture-, PeCDD- and HxCDD-treated groups. Other effects elicited by exposure to the mixture included: decreased liver tryptophan 2,3-dioxygenase (TdO) activity, increased serum tryptophan concentrations, and decreased concentration of serum glucose. Positive control group responses followed a pattern similar to that of the mixture-treated groups.

In a follow-up experiment using the same experimental conditions, Viluksela *et al.* (1998b) reported a dose-dependent weight gain reduction in mixture-treated rats. The authors concluded that TEFs derived from acute studies could be used to predict the toxicity of mixtures of PCDDs regardless of whether they are administered as single compounds or as a mixture. Their results confirmed the notion of additive toxicity for the PCDDs and validated the TEF methodology (Viluksela *et al.*, 1998a; 1998b).

Gao *et al.* (2000) exposed gonadotropin-primed immature female rats (23-day old) to individual congeners: 2,3,4,7,8-PeCDF, PCB 126 and PCB 52, or a mixture of PCDDs. The PCDD mixture included: TCDD, PeCDD, and HxCDD in addition to PeCDF and PCB 126. Equine chorionic gonadotropin (eCG; 5 IU) was injected 24 h after dosing to induce follicular development. At the day of expected ovulation, 72 h after eCG injection, treatment with the individual congener PeCDF, PeCB and/or their mixture with PCDDs generated parallel dose-response curves for the inhibition of the eCG-induced ovulation. Serum concentrations of 17- β -estradiol (E2) were increased by PeCDF, PCB 126 and the mixture. In contrast, serum progesterone (P4) and follicle stimulating hormones (FSH) were decreased at that same time point. Ovarian histology revealed ova in large preovulatory follicles and a lack, or a reduced number, of corpora lutea for rats treated with PeCDF, PCB 126 and the mixture. These histological effects were very similar to those observed in PCDD-treated rats (Gao *et al.*, 1999). The authors concluded that these findings and the similarity in the slope of the dose-response relationships for the individual congeners (PeCDF and PCB 126) and their equipotent mixture with PCDDs support the concept of TEQ for the inhibition of ovulation (Gao *et al.*, 2000).

van der Plas *et al.* (2001) exposed female SD rats to a complex mixture of DL-PHAHs covering more than 90% of the total TEQs contaminants present in Baltic herring. The severe decrease in hepatic retinoid level observed in the rats treated with the DL-PHAH mixture was similar to the effect of a TEQ equivalent dose of 1 µg 2,3,7,8-TCDD/kg body weight (bw)/week. However, plasma retinol decrease could not be predicted using the TEF concept. Treatment with the DL-PHAH mixture decreased plasma retinol by 21 % whereas an increase of 21 % was observed in TCDD-treated rats. Total plasma thyroid hormone exhibited a more severe decrease in the PHAH mixture-treated group when compared to the TCDD-treated group (~60% vs 38 %). The authors noted that the discrepancy between the observed and the predicted effects for plasma retinol and thyroid hormone levels could be attributed to the additional effect of hydroxylated PCBs formed by metabolism of PHAHs present in the mixture.

Additionally, van der Plas *et al.* (1999) exposed female SD rats to a laboratory-derived mixture of PHAHs in a medium term two-stage initiation/promotion bioassay. The PHAH mixture containing 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, PCB 126, PCB 118, PCB 156, and PCB 153 represented more than 90% of the total TEQ contaminants present in Baltic herring. Diethylnitrosamine injection (30 mg/kg bw ip) 24 h after a partial hepatectomy was used as an initiator. Starting six weeks after initiation, rats were given subcutaneous injections of the PHAH mixtures, alone or in combination with PCB 153, a di-*ortho* substituted PCB, weekly for 20 weeks. PHAH treatment caused liver enlargement and increased activity in hepatic cytochrome P450 1A1, 1A2, 2B1 and 2B2. In addition, rats dosed with 1 µg TEQ PHAH mixture/kg bw/week exhibited a significantly lower volume fraction of liver occupied by foci in comparison to the TEQ equivalent TCDD-dosed group (3.8 and 8.7%, respectively). When the PHAH mixture was administered in combination with PCB 153, the volume fraction of liver with foci was significantly increased by 0.5, 1 and 2 µg TEQ/kg body wt/week (4.5, 5.2 and 6.6%, respectively) compared to the control group (2% of liver volume containing foci). However, this increase was less than the increase expected based on the TEQ doses. The authors concluded that the TEQ-based administered dose overestimated the observed tumor-promoting effects of the PHAH mixture by a factor of two. Nevertheless, the authors noted that their results support the assumption of additivity for the TEF methodology (van der Plas *et al.*, 1999).

Groups of five adult female SD rats were gavaged with 0, 2.5, 25, 250 or 1000 ng TCDD/kg bw/day or TCDD in combination with a PCB mixture at 2 or 20 µg/kg bw/day for a period of 28 days (Wong *et al.*, 1997). Rats treated with either 1000 ng TCDD alone or the mixture of 1000 ng TCDD + 2 µg PCBs showed growth suppression, increased absolute and relative liver weights and decreased thymic weight. Of these three TCDD-induced responses, only growth suppression appears to be altered by co-administration of TCDD and PCBs. Growth suppression appeared to be more pronounced in the group receiving 1000 ng TCDD + 2 µg PCBs than the one receiving TCDD alone. Hepatic microsomal methoxyresorufin-O-demethylase (MROD) and EROD activities were significantly increased in 250 and 1000 ng TCDD-treated rats, but co-administration of PCBs antagonized these responses. Similarly, co-administration of 20 µg PCBs and 250 ng TCDD elicited statistically significant antagonistic effects on serum cholesterol and on liver UDP glucuronyl transferase activity and ascorbic acid. On the other hand, co-administration of 1000 ng TCDD with PCBs did not affect the following responses: increased serum albumin, decreased liver vitamin A, and

increased kidney vitamin A and liver microsomal glutathione-S-transferase activity. Based on these results, the authors concluded that the effects of the PCB mixture and TCDD may be additive or antagonistic depending on the dose level and end points measured. Knowledge of mechanisms of actions and toxicokinetics is therefore required to predict toxicity for mixtures containing PCBs (Wong *et al.*, 1997). The discrepancy in the effects may be partly explained by the fact that the typical PCB mixture contains a substantial proportion of the *ortho*-PCBs that are inactive in terms of DL-toxicity, but are well-known to have other significant effects, including an anti-thyroid hormone action. It is worth noting that PCB commercial mixtures may vary greatly, from lot to lot, in the proportion of *ortho*-PCBs (Burgin *et al.*, 2001; Kodavanti *et al.*, 2001).

Körner *et al.* (2001) compared the inductive potency of PCDD and PCDF mixtures to that of TCDD in Wistar rats treated 16 times (every 3rd day) subcutaneously with a defined mixture. Each single dose of the PCDD mixture and PCDF mixture was calculated to contain 57 or 39 ng I-TEQ/kg bw, respectively. Both mixtures contained a large excess of non-2,3,7,8-substituted congeners. Based on EROD induction in rat hepatic microsomes, the authors reported a 3 to 4-fold overestimation of the I-TEQ for the concentration range tested in this study. Based on liver concentration of the mixture, the concentration-response curves for both the PCDD and PCDF mixtures run parallel to the curve for 2,3,7,8-TCDD. Körner *et al.* (2001) explained the discrepancy in potency between the mixtures and TCDD by the differential kinetic, tissue distribution, and elimination half-life of the various congeners.

DeVito *et al.* (2000) dosed mice, by gavage, with either TCDD, PCB 105, PCB 118, PCB 126, or PCB 156, five days/week for 13 weeks. Based on the EROD activity in liver, lung and skin and the acetanilide-4-hydroxylase activity in liver, the relative potency for these congeners varied across end points by a factor of less than 4 when calculated based on an administered dose. In general, for every chemical, the relative potency for at least one response differed by an order of magnitude or more from the other responses. The authors concluded that the relative potencies may be dose-dependent (DeVito *et al.*, 2000).

Harper *et al.* (1995) investigated the immunosuppressive effect of several PCB mixtures and congeners on the splenic plaque-forming cell (PFC) response and serum IgM units to the antigen, trinitrophenyl-lipopolysaccharide in female B6C3F1 mice. The ED₅₀ values for Aroclor 1260-, 1254-, 1248- and 1242-induced immunotoxicity varied by less than 2-fold from 355 to 699 mg/kg. The immunotoxicity-derived TEFs for TCDD, and some chlorobiphenyl compounds (PCB 77, PCB 126, PCB 169, PCB 105, PCB 118, PCB 157, PCB 189, PCB 170, and PCB 180) could be calculated from the ratios ED₅₀-(TCDD)/ED₅₀-(congener). The TEF values were within the range of those previously determined for other AhR-mediated responses. Based on the known concentrations of these congeners in the PCB mixtures, TCDD or TEQs in the mixture were calculated [i.e., TEQ = Σ (PCB congener x TEF)] using the immunotoxicity-derived TEFs (plaque-forming cells/10⁶ viable cells). TEQ values for Aroclors 1260, 1254, 1248, and 1242 were 16.0, 54.4, 260.4, and 197 ppm, respectively. Based on the ED₅₀ value for the immunosuppressive activity of TCDD (4.8 µg/kg), the calculated ED₅₀ values for immune suppression by Aroclors 1260, 1254, 1248, and 1242 were 300, 88, 18, and 24 mg/kg, respectively. The ED₅₀ (observed)/ED₅₀ (calculated) ratios were 1.2, 5.9, 21, and 22.0 for Aroclors 1260, 1254, 1248, and 1242, respectively (Harper *et al.*, 1995).

DL-PCBs (the non-*ortho*- and mono-*ortho*-substituted congeners) elicit *AhR*-mediated responses. Studies with mixtures of PCB 126 or PCB 156 and TCDD found little deviation from additivity. The additivity assumption was further confirmed by early life stage fish studies with binary mixtures of PCDDs, PCDFs, and PCBs congeners or complex mixtures of DL- and non-DL PCDDs, PCDFs and PCBs at environmentally relevant dose ratios and dose levels (Walker and Peterson, 1991; Walker *et al.*, 1996; Zabel *et al.*, 1995). The validity of the additivity assumption was assessed by determining the significance of interactions between pairs of PCDD, PCDF, and PCB congeners when injected into newly fertilized rainbow trout eggs in ratios relevant to those found in feral lake trout eggs from the Great Lakes (Zabel *et al.*, 1995). Most of the congener pairs tested acted additively in causing rainbow trout early life stage mortality. Table 2 shows the dose ratio of PCDD, PCDF, and PCB congener pairs injected into rainbow trout eggs and the ratio of their concentrations in feral lake trout eggs. However, TCDD/PCB 77 and TCDD/PCB 126 showed evidence of a statistically significant interaction that deviated from additivity. From these data, Zabel *et al.* (1995) concluded that the use of fish-specific TEFs might not exactly predict the mortality risk posed to fish early life stages by the mixture of TCDD-like congeners in the eggs. Nevertheless, the relatively small deviation (1 to 4-fold) from additive interaction in this study warrants the additivity assumption in assessing the risk to fish early life stage mortality posed by TCDD and related compounds in eggs.

Walker *et al.* (1996) examined the TEQ additive properties of 11 TCDD-like congeners and three non-TCDD-like congeners combined at ratios typically found in Lake Michigan lake trout. Early life stage mortality elicited by the mixture or TCDD alone exhibited parallel dose-response curves in lake trout and rainbow trout. Based on LD₅₀ values, the dose-response curves for the mixtures were significantly shifted to the right of the TCDD dose-response curves by 1.3 and 1.8-fold for lake trout and rainbow trout, respectively. The data suggest that TCDD-like congeners, although acting through a common mechanism of toxicity in early life development in fish, may not act strictly additively when combined in a mixture of TCDD- and non-TCDD-like congeners at ratios found in Great Lakes fish (Walker *et al.*, 1996). However, Walker *et al.* (2005) evaluated the TEF approach in the 2-year rodent (female Harlan SD rats) cancer bioassays with TCDD, PCB 126, PeCDF, or a mixture of the three compounds. Statistically based dose-response modeling indicated that the shape of the dose-response curves for hepatic, lung, and oral mucosal neoplasms was the same in studies of the three individual chemicals and the mixture. The dose response for the mixture could predict a combination of the potency-adjusted doses of the individual compounds. By using the WHO-97 TEF values, they successfully predicted the increased incidence of liver tumors (hepatocellular adenoma and cholangiocarcinoma) that was induced by exposure to the mixture. Therefore, their data support the use of the TEF approach for dioxin cancer risk assessments (Walker *et al.*, 2005).

Thus, the predominance of additive interactions between PCDDs, PCDFs, and DL-PCBs supports the concept of TEF. This is true for various species of fish, birds and mammals exposed to congener dose ratios and congener levels at environmentally relevant doses (Safe, 1990; 1994; Cook *et al.*, 1997; van den Berg *et al.*, 1998; van den Berg *et al.*, 2006).

UNCERTAINTIES ASSOCIATED WITH THE USE OF THE TEF METHODOLOGY

Quantifying uncertainty surrounding the TEF estimate is difficult. TEF estimates are generated from several sources of experimental data and for some congeners can vary by several orders of magnitude. This apparent variability has been attributed to different exposure regimens, test species, or purity of the test compound (US EPA, 2000). For tests involving exposure to commercial mixtures of PCBs such as Aroclor 1254, significant differences in the composition profile of PCB congeners (Kodavanti *et al.*, 2001), and toxicological responses (Kodavanti *et al.*, 2001; Burgin *et al.*, 2001) were reported to exist between lots.

For the WHO TEF 2005 reevaluation process, the refined TEF database published by Haws *et al.* (2006) was used as a starting point, which will facilitate better characterization of the variability and uncertainty inherent in the data (Haws, *et al.* 2006). Decisions about a TEF value were made based on a combination of unweighted relative effect potency (REP) distributions from this database, expert judgment, and point estimates (Van den Berg, *et al.* 2006).

It is worth noting that TEF estimates are point estimates. They are derived from scientific judgment based on examination of REP for various end points. However, these semi-quantitative judgments are made in the context of risk assessment, and provide valuable insight in the estimation of TEQs.

Variability in estimated REPs for individual congeners may or may not significantly impact risk estimates, which depend on a congener's TEF value, and the amount inside the body or exposure environment, among other factors. More importantly, the most sensitive animal species should be used in risk assessment to protect susceptible human populations. For example, using WHO-97 TEF values (van den Berg *et al.*, 1998) to look at background exposure from a typical U.S. diet, it is clear that only a limited number of congeners significantly contribute to the total TEQ. More than 60% of the TEQ_{WHO-97} associated with background dietary exposure (1 pg/kg/d) is attributable to only four congeners: 2,3,7,8-TCDD (8%), 1,2,3,7,8-PeCDD (21.5%), 2,3,4,7,8-PeCDF (10.7%), and PCB 126 (21%) (US EPA, 2000). The variability in the REP values reported in the literature for these congeners is much lower than for other congeners that contribute minimally to background TEQ. Since the TEFs for the major congener constituents of background exposure (or other exposure with a similar congener profile) have consistently been determined empirically to be within a factor of 2-3, it is therefore unlikely that the estimated TEQ overestimates the "true" TEQ by more than a factor of five (US EPA, 2000). Moreover, non-DL-PCBs at background level are unlikely to significantly affect the uncertainty of TEQ estimates as discussed later in this section.

The assumption of additivity is essential in the TEF approach. Although antagonistic and/or synergistic interactions are seen at some dosage ratios and doses of congeners for specific toxicity end points, these types of interaction are seldom observed. Rather, additivity appears to be the most common interaction reported in the scientific literature describing *AhR*-mediated chemical toxicity.

Criticisms concerning the TEF approach mainly focus on four areas (van den Berg *et al.*, 2000):

1. Non-additive interaction of DL-congeners when there is co-exposure to non-DL-congeners, particularly PCB 153
2. Differences in species responsiveness
3. Differences in the shape of the dose-response curves between individual *AhR* agonists
4. Mono-*ortho* PCBs in the TEF concept

Non-additive interactions

Non-additive interactions in mixtures containing both PCDDs/Fs and specific *ortho*-substituted PCBs such as PCB 153 (a di-*ortho* PCB representing a major environmental contaminant) have been observed in laboratory studies (Safe, 1997). Several "non-dioxin-like" PCBs, including PCB 153 and commercial PCBs exhibit "anti-dioxin" or *AhR* antagonist activity (Biegel *et al.*, 1989; Davis and Safe, 1989, 1990).

Commercial PCB mixtures such as Aroclor 1254 and PCB 153 inhibit TCDD-induced immunotoxicity and fetal cleft palate (teratogenicity) in C57BL/6J mice, an *Ah*-responsive strain (Biegel *et al.*, 1989). PCB 153 at doses as high as 750 to 1000 $\mu\text{mol/kg}$ did not cause fetal cleft palate, suppress the splenic plaque-forming cell response to sheep red blood cells, or induce hepatic microsomal EROD in C57BL/6J mice. Co-treatment of TCDD and PCB 153 or Aroclor 1254 in mice partially antagonized these *AhR*-mediated responses to TCDD (Biegel *et al.*, 1989). The authors observed a competitive binding of the antagonists for the *AhR*.

Moreover, Davis and Safe (1989) have demonstrated a significant antagonistic interaction in mice treated with commercial PCB mixtures Aroclors 1260, 1254, 1248, 1242, 1016 (25 mg/kg dose) and with reconstituted PCB mixture (resembling a PCB extract from human milk; 50 mg/kg) and with TCDD (3.7 nmol/kg). The interaction significantly antagonized the TCDD-mediated inhibition of the splenic plaque-forming cell response in C57BL/6J mice.

Zhao *et al.* (1997) reported similar results. They compared the toxicity of PCB 126 to that of co-treatment with PCB 126 and PCB 153 for the following end points: the induction of fetal cleft palate in offspring from C57BL/6 mice, the inhibition of splenic plaque-forming cell (PFC) response and the decrease serum IgM levels. Zhao *et al.* (1997) reported an antagonistic interaction between PCB 153 and PCB 126.

Non-additive interactions between PCB 153 and DL-PCBs such as PCB 126 and PCB 169 were also reported by Harper *et al.* (1995) in female B6C3F1 mice for the inhibition of the splenic plaque-forming cell (PFC) response and serum IgM units to the antigen, trinitrophenyl-lipopolysaccharide.

Wolfe (1998) used *in vitro* assays of transformation of carcinogen-initiated C3H/M2 mouse fibroblasts to study interactions of mixtures. They reported an additive promoting effect of a

defined mixture of PCB126 and TCDD; but PCB 153 antagonized the TCDD-mediated promotion. The authors concluded that the TEF-approach may be insufficient to estimate the tumor-promoting activities of PCDDs, PCDFs, and PCBs in mammalian tissues in which di-*ortho* substituted PCBs are greatly accumulated.

Thus, non-additive interactions between different classes of PCBs present in environmental samples suggest that the TEF approach may overestimate the effective TEQ for some responses in animal models (Safe, 1998). Safe (1997) noted in his review that the deviations from additive interaction reported in the literature were associated with a lack of data on the actual tissue concentration or body burdens of the congeners. The deviation from additivity could also be associated with exposure to *ortho*-PCBs, particularly with PCB 153 in combination with PCDDs and PCDFs.

With respect to the non-additive interaction between TCDD and PCB 153 on CYP1A1 induction, it was observed that at lower dose levels the interaction appears to be synergistic, while at the higher dose levels antagonistic effects were observed (van den Berg *et al.*, 1994). Mechanistically, the antagonistic interaction was explained by the competition for binding to the AhR of the compound (less potent) with a low binding affinity for the AhR (Astroff *et al.*, 1988; Biegel *et al.*, 1989). Similarly, additive interaction with a mixture of DL-PCBs is hindered when increasing concentrations of non-DL-PCBs are added to the mixture (Schmitz *et al.*, 1995). For liver tumor promotion in rodents, prediction of this effect is complicated by the dose-dependent accumulation of PCDDs, PCDFs, and DL-PCBs in the liver (Tritscher *et al.*, 1991; Mills and Andersen, 1993).

Pharmacokinetic interaction between PCBs has also been reported. Lee *et al.* (2002) observed an increased PCB 153 retention in the liver and a decreased PCB 153 accumulation in the fat of nonpregnant C57BL/6 mice coadministered PCB 153 (20 mg/kg) and PCB 126 (0.2 mg/kg). However, little or no significant pharmacokinetic interactions were observed in lactating mice and suckling pups. The shift of the accumulated PCB 153 from fat to liver could possibly be linked to the induction of CYP1A2 protein by PCB 126. TCDD and 2,3,4,7,8-PeCDF were shown to be sequestered mostly in the liver in mice expressing CYP1A2 gene (Diliberto *et al.*, 1999). This disposition pattern of TCDD and 2,3,4,7,8-PeCDF was in contrast with the one observed in knockout mice lacking CYP1A2 expression.

Differences in species responsiveness

Species differences in the functional responses to TCDD and related DL-compounds could be important (Peterson *et al.*, 1993). Van den Berg *et al.* (2000) proposed several factors to explain species differences in response to AhR agonists. These factors include toxicokinetics, receptor distribution and affinity, agonistic action on receptor upon binding, etc. However, most biological effects caused by DL-compounds occur at levels of DL-compounds that differ by less than one order of magnitude between species (DeVito *et al.*, 1995). There is a large difference between species in the pharmacokinetics of TCDD and related compounds. In addition, liver/adipose tissue distribution can vary significantly among species and dose levels used. Highly potent congeners such as 2,3,4,7,8-PeCDF, 2,3,7,8-TCDD and PCB 126 accumulate in the liver because of their tight binding to the CYP1A2 enzyme (DeVito *et al.*, 1995; DeVito and Birnbaum, 1995; DeVito *et al.*, 1995; Diliberto *et al.*, 1997). This feature

is more pronounced in rodents than humans, with monkeys being intermediate (van den Berg *et al.*, 2000). The non-linear hepatic accumulation disappears at lower dose levels (10 ng TCDD/kg) (Abraham *et al.*, 1988). Similar findings were observed in humans in the case of the Yusho incident. Highly exposed individuals had a liver/adipose tissue ratio 2 to 3 orders of magnitude higher than that of individuals exposed to background levels of 2,3,4,7,8-PeCDF. Thus, it seems that the non-linear hepatic accumulation of PCDDs/PCDFs observed in rodent studies only occurs at dose levels used to determine relative potencies of PCDDs and PCDFs. Such nonlinear hepatic accumulation of PCDDs/PCDFs is unlikely to occur in humans exposed to these chemicals at background levels (van den Berg *et al.*, 2000). Differences in tissue distribution can significantly influence TEF values, when they are based on tissue concentrations (DeVito and Birnbaum, 1995).

Several authors showed, from studies in various human cell types and tissues, that human AhRs have a range of binding affinities for TCDD generally below but overlapping that observed in rodent strains (Harper *et al.*, 1986; 1988; Roberts *et al.*, 1986; Lorenzen and Okey, 1991). However, CYP1A1 inducing activities of TCDD and PCDD congeners were found to be 10-fold less potent in human primary hepatocytes and HepG2 cells than in the respective rat model (Lipp *et al.*, 1992; Schmitz *et al.*, 1995). On the other hand, the effectiveness of inducers of CYP1A1 activity in human and rat hepatoma cells differed only moderately (Lipp *et al.*, 1992), and human and rat thymus transplanted into severe combined immunodeficient (SCID) mice showed similar sensitivity to TCDD immunotoxicity (Vos *et al.*, 1998).

In general, the binding affinity data of different AhR ligands has limited usefulness as a predictor of agonist activity. Induction potency of CYP1A1 in cell culture for a number of AhR ligands was poorly correlated with AhR binding affinity (Santostefano *et al.*, 1992). Rather, the DNA binding form of the AhR seems to be a better predictor of AhR agonist potency.

Differences in the shape of the dose-response curves for individual Ah receptor agonists

Different agonists for the AhR exhibit different dose-response curve shapes. For instance, the maximal efficacy of OCDD as an inducer of CYP1A1 is much lower than that of TCDD in rat primary hepatocytes (Schrenk *et al.*, 1991). Taking into account the numerous AhR independent factors involved in AhR-mediated toxicity, different slopes of the dose-response curves for DL-compounds are to be expected (van den Berg *et al.*, 2000). However, for tests *in vitro* where toxicity end points are linked to AhR activation in a relatively simple fashion, dose-response slopes for potent PCDDs and PCDFs are generally reported to be similar. Induction of CYP1A1 activity in hepatocytes by DL-PCBs generates dose-response curves with similar slopes. However, such is not the case for PCB 77, since this congener is readily metabolized. Also, relatively high amounts of non-DL-PCBs lead to alterations in slope of the concentration-response curve of the mixture (Schmitz *et al.*, 1995).

Mono-ortho PCBs in the TEF concept

Mono-ortho PCBs represent a particular case since these congeners can elicit end points such as carcinogenicity, porphyrin accumulation, and alterations in circulating thyroid hormone

concentrations (Khan *et al.*, 2002). Moreover, neurotoxicity in mammalian species could arise by both *AhR*-mediated and non-*AhR*-mediated mechanisms (van den Berg *et al.*, 2000). Di-, tri-, and tetra-*ortho* PCBs can also share the non *AhR*-mediated pathway, which introduces more uncertainty in the risk assessment especially when considering end points common to both of these pathways (van den Berg *et al.*, 2000). Thus, end points with clearly recognized *AhR*-mediated mechanisms should be selected for the determination of TEF values when mono-*ortho* PCBs are involved.

However, NAS (2006) reported that “Overall, even given the inherent uncertainties, the toxic equivalency factor (TEF) method provides a reasonable, scientifically justifiable, and widely accepted method to estimate the relative toxic potency of DLCs on human and animal health. However, the reassessment should acknowledge the need for better uncertainty analysis of the TEF values.” (<http://www.ejnet.org/dioxin/nas2006.pdf>).

IMPLICATION OF THE NEW TEF METHODOLOGY

The TEF methodology is the best available tool for the health risk assessment of complex mixture of DL-chemicals. Assuming dose-additivity of the various components of a chemical mixture, it uses the toxicological equivalent mass of TCDD to evaluate risk. Clearly, to consider each component of a chemical mixture as having the toxicological potency of TCDD would be overestimating the potential health risk of the mixture. The use of the TEF method allows for a more accurate estimate of the health risks. However, in using the TEF methodology, one must bear in mind that although the various congeners of a mixture have relative equivalent toxicity to TCDD, these congeners do not necessarily share the same environmental fate as TCDD. Consequently, the profile of chemical constituents in a mixture could change as the released mixture moves away from its source and as it ages over time. Also, other chemicals such as di-*ortho* PCBs, not eliciting toxicological effects through the *AhR*-mediated pathway, and endogenous DL-compounds, not included in the TEF methodology, might bias the risk assessment estimate obtained from the TEF methodology. Thus, improvements to the TEF methodology should include risk assessment methods considering not only *AhR*-mediated toxicological responses but also those mediated by other toxicological pathways.

Many criteria for regulation of PCDD/PCDF contamination are based on TEQs. These values include emission limits for industrial plants, tolerable daily intake (TDI) and environmental quality standards. However, the use of different TEF schemes, or even the absence of TEQ calculations, in the available databases makes the interpretation and comparison of criteria and regulation quite difficult.

There may be changes to the total TEQ estimates from a variety of sources depending on the TEF scheme used. Switching from the I-TEF to the TEF_{WHO-97} could result in an increase in estimated TEQ of 1-10% for the emissions to air and 10-20% increase in food, but a substantial decrease (up to 70%) in sludge samples (Dyke and Stratford, 2002).

The WHO-97 TEF values for the mono-*ortho* PCBs ranged from 0.00001 to 0.0005 and a major issue was that the REP values for the different mono-*ortho* PCBs span four to five orders of magnitude (van den Berg *et al.*, 1998). In 2005, the WHO panel considered possible

inconsistent and low level contamination of the mono-*ortho* PCBs with more potent DL-compounds to play, at least in part, a role in causing this large variation. They therefore recommended that the TEFs of three most-environmentally relevant mono-*ortho* PCBs 105, 118, and 156 be set at 0.00003 based on their medians of the REP distribution. A differentiation for all other remaining mono-*ortho* PCBs was considered not feasible due to the lack of sufficient experimental data and thus they recommended WHO-05 TEFs for all mono-*ortho* PCBs be set at 0.00003 (van den Berg *et al.*, 2006). In addition, they adjusted WHO-97 TEF values (0.5) to half-log values (0.3). Overall, the values of the WHO-05 TEFs for 13 congeners were revised (5 values go up and 8 values go down) and total concentrations of dioxin equivalents in dietary exposure are thereby reduced about 10-20% (van den Berg *et al.*, 2006; De Mul *et al.*, 2008). The percentage reductions in total TEQ levels calculated for the same biotic samples by using WHO-05 TEFs, rather than WHO-97 TEFs, are shown in Figure 2.

Patterson *et al.* (2008) reported the reference ranges for the total TEQ and TEQ sub-fractions of PCDDs, PCDFs, coplanar PCBs, and mono-*ortho*-PCBs in a statistically designed sampling of the US population in 2001-2002. They found about 80-90% of the total TEQ can be estimated by using seven congeners, i.e., 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD, 2,3,4,7,8-PeCDF, PCB 126, PCB 118, and PCB 156. The TEQ levels are lower using the WHO-05 TEFs compared to using WHO-97 TEF values, which are principally due to the much lower TEF_{WHO-05} values assigned to the mono-*ortho*-PCBs. They also found that geometric mean TEQ levels in pooled samples from the US population increased with age. In the youngest age group (12-19 years), the TEQ levels were higher in males than in females while females had higher TEQ levels than males in all older age groups. In the pools, as age increases the percent contribution of the PCDD TEQ levels increases while the percent contribution of the PCDF TEQ levels decreases for all race/ethnicity and sex strata. The TEQs in pg/g lipid for all individuals ≥ 20 years old in the US population are shown in Table 3 (Patterson *et al.* 2008).

Hong *et al.* (2009) used a major exposure study which examined blood, household dust, and soil levels of DL-compounds in several regions of Michigan to compare total TEQ in various media using WHO-97 and WHO-05 TEFs. They found the mean total TEQ was significantly reduced by 26%, 12% and 14% for serum, household dust, and soil, respectively, when the TEQ was based on the WHO-05 TEFs compared to the WHO-97 TEFs. They noted that decrease in the serum total TEQ was largely due to the down-weighting of the TEFs for the majority of mono-*ortho* PCBs. In contrast, the decrease in the soil total TEQ was mostly due to the down-weighting of the TEF for 2,3,4,7,8-PeCDF (WHO-97 TEF = 0.5, WHO-05 TEF = 0.3). For household dust, the decrease in total TEQ was not due to any single TEF but was due to small changes in a number of compounds (Hong *et al.* 2009).

Bhavsar *et al.* (2008) compared TEFs that have been developed by various agencies over 25 years. Their results from consumption of fish showed that the mammalian PCDD/F-TEQ based on WHO-05 TEF is about 7.5% lower than that based on WHO-97 TEF. The mammalian WHO-05 DL-PCB TEQ is on average 25-26% lower than WHO-97 DL-PCB TEQ. Total WHO-05 TEQ is on average 22% lower than WHO-97 total TEQ. According to the WHO-05 toxicological standards for dioxins/furans, all previous major TEF schemes except Germany-85 TEF and US EPA-87 TEF were conservative (i.e., higher) in estimating

TEQs. The major (>75%) contribution to WHO-05 PCDD/F-TEQ is from 2,3,7,8-TCDD (33%), 1,2,3,7,8-PCDD (26%), 2,3,7,8-TCDF (10%), and 2,3,4,7,8-PCDF (9%). The WHO-05 DL-PCB TEQ is dominated by PCB 126 which on average contributes about 88%. The DL-PCB TEQ generally contribute >70% of total TEQ. The author recommends that the congener-specific concentrations, TEF scheme, and names of compounds be presented whenever reporting TEQs (Bhavsar *et al.* 2008).

A statistically based survey of dioxins and DL-compounds in domestic meat and poultry was conducted by the U.S. Department of Agriculture (USDA) from September 2007 to September 2008. Seventeen toxic polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and four non ortho-polychlorinated biphenyls (no-PCBs) were measured in 510 beef (steer/heifer), market hog, young turkey, and young chicken samples. The results of the survey showed the sum of PCDD/F and non-ortho-PCB toxic equivalencies (sum-TEQs) ranged from not detect to 4.5 pg/g of lipid. Mean sum-TEQ levels for beef, turkey, chicken, and pork were 0.66, 0.61, 0.17, and 0.16 pg/g of lipid, respectively. To compare the new survey data with data from previous USDA surveys in the mid-1990s and 2002-2003, TEQs from all data sets were calculated using the most recent 2005 TEFs. The results of the recalculation on the older survey data was a small increase (4-13%) in mean TEQs for the mid-1990s data, which initially used pre-1994 TEFs, and a small decrease (2-4%) for the 2002-2003 data, which initially used 1998 TEFs (Huwe *et al.* 2009).

California data were also considered to estimate the consequences of using the TEF_{WHO-97} and TEF_{WHO-05} values rather than the I-TEF scheme (Table 4). These results are in agreement with findings reported by Dyke and Stratford (2002). Total TEQ PCDD/F emission measured in air increased by more than 10% when the TEF_{WHO-97} and TEF_{WHO-05} schemes were used in place of the I-TEF. A 500% increase in total TEQ was calculated for fish (striped bass) from the San Francisco Bay using TEF_{WHO-97} and TEF_{WHO-05} rather than the I-TEF scheme. This difference is mostly attributable to the inclusion of PCB congeners in the TEQ_{WHO-97} and TEF_{WHO-05} calculation (0.60 versus 3.45 and 3.15 pg/g TEQ as calculated according to the I-TEF, TEF_{WHO-97} and TEF_{WHO-05} schemes, respectively (Table 5). Values calculated using TEF_{WHO-05} are typically close to, but slightly lower than, those estimated using TEF_{WHO-97} , depending on the exact distribution of congeners present.

CONCLUSION

The TEF/TEQ methodology is the best available method for assessing risk of dioxin and DL-compounds, although some limitations exist. OEHHA has used it in the Technical Support Document for Cancer Potency Factors for many years and will continue to use this current version until the next update or extension of the methodology appears. Considerable amounts of data have been generated by using TEF method and have been found to be very useful for risk assessment. However, the exclusion of non-DL-compounds from the TEF methodology represents perhaps the most obvious limitation of the TEF approach (Yang *et al.* 2010). The TEF approach has been adopted by interested parties on condition that the TEF methodology remains an interim method and that it should be reevaluated periodically. Clearly, the ultimate goal should aim to include both cancer and non-cancer effects of non-DL-compounds in order to have a more accurate estimate of the health risk caused by these persistent and bioaccumulative chemicals. At this point, it is important for public health protection that the

most scientifically relevant and up-to-date TEF methodology be used. Therefore, we propose to use the TEF_{WHO-05} table of equivalency values in place of the previous I-TEF or TEF_{WHO-97} versions in risk assessments conducted under the Air Toxics Hot Spots program. The TEF_{WHO-05} values are based on the latest scientific findings available, and like the previous TEF_{WHO-97} values include DL-PCBs as well as dioxins, all of which contribute to the total TEQ concentration of a sample. It also facilitates the comparison of environmental measurements to other international databases.

Table 1. TEF values used or proposed in California

Congener	California TEF ^a	I-TEF ^b	TEF _{WHO-97} ^c	TEF _{WHO-05} ^d
PCDDs				
2,3,7,8-TCDD	1	1	1	1
1,2,3,7,8-PeCDD	1	0.5	1	1
1,2,3,4,7,8-HxCDD	0.03	0.1	0.1	0.1
1,2,3,6,7,8-HxCDD	0.03	0.1	0.1	0.1
1,2,3,7,8,9-HxCDD	0.03	0.1	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.03	0.01	0.01	0.01
1,2,3,4,6,7,8,9-OCDD		0.001	0.0001	0.0003
PCDFs				
2,3,7,8-TCDF	1	0.1	0.1	0.1
1,2,3,7,8-PeCDF	1	0.05	0.05	0.03
2,3,4,7,8-PeCDF	1	0.5	0.5	0.3
1,2,3,4,7,8-HxCDF	0.03	0.1	0.1	0.1
1,2,3,6,7,8-HxCDF	0.03	0.1	0.1	0.1
1,2,3,7,8,9-HxCDF	0.03	0.1	0.1	0.1
2,3,4,6,7,8-HxCDF	0.03	0.1	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.03	0.01	0.01	0.01
1,2,3,4,7,8,9-HpCDF	0.03	0.01	0.01	0.01
1,2,3,4,6,7,8,9-OCDF		0.001	0.0001	0.0003
PCBs (IUPAC #, Structure)				
77 3,3',4,4'-TCB			0.0001	0.0001
81 3,4,4',5-TCB			0.0001	0.0003
105 2,3,3',4,4'-PeCB			0.0001	0.00003
114 2,3,4,4',5-PeCB			0.0005	0.00003
118 2,3',4,4',5-PeCB			0.0001	0.00003
123 2',3,4,4',5-PeCB			0.0001	0.00003
126 3,3',4,4',5-PeCB			0.1	0.1
156 2,3,3',4,4',5-HxCB			0.0005	0.00003
157 2,3,3',4,4',5'-HxCB			0.0005	0.00003
167 2,3',4,4',5,5'-HxCB			0.00001	0.00003
169 3,3',4,4',5,5'-HxCB			0.01	0.03
170 2,2',3,3',4,4',5-HpCB			0	-
180 2,2',3,3',4,4',5,5'-HpCB			0	-
189 2,3,3',4,4',5,5'-HpCB			0.0001	0.00003

■ = Value introduced or changed

^a CDHS, 1986. ^b NATO/CCMS, 1989. ^c van Leeuwen, 1997. ^d Van den Berg, 2006.

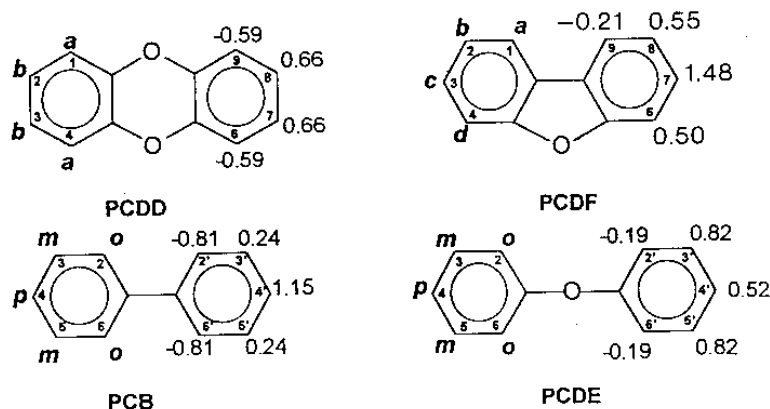
Table 2. Dose ratio of PCDD, PCDF, and PCB congener pairs injected into rainbow trout eggs and the ratio of their concentrations in feral lake trout eggs

Congener pair		Ratio of congener concentrations in feral lake trout eggs in 1991^a		Ratio of congener doses injected into rainbow trout eggs^b
Congener 1	Congener 2	Lake Michigan Congener 1/2	Lake Ontario Congener 1/2	Congener 1/2
1,2,3,7,8-PCDD	TCDD	1.4:1	0.67:1	7:1, 2:1, 1:1, 0.3:1
2,3,4,7,8-PCDF	1,2,3,7,8-PCDD	4.7:1	1.2:1	3:1, 1.3:1, 0.5:1
2,3,7,8-TCDF	2,3,4,7,8-PCDF	1.5:1	1:1	53:1, 20:1, 9:1, 3:1
2,3,4,7,8-PCDF	TCDD	3.2:1	0.77:1	10:1, 4:1, 0.6:1
PCB 77	PCB 126	3.7:1	3:1	88:1, 32:1, 15:1, 5:1
PCB 105	TCDD	38,000:1	1,600:1	10,000:1, 6000:1, 3000:1, 1000:1
PCB 153	TCDD	116,000:1	NA	5000:1, 1000:1, 500:1, 100:1
PCB 126	TCDD	800:1	60:1	400:1, 200:1, 74:1
PCB 77	TCDD	3,000:1	167:1	30,000:1, 4500:1, 3500:1, 1250:1
PCB 105	PCB 126	47:1	27:1	25:1
PCB 118	PCB 126	88:1	112:1	51:1

^a Lake Michigan and Lake Ontario ratios (Congener 1/Congener 2) were derived from congener-specific high resolution gas chromatograph/high resolution mass spectrometry performed on lake trout egg samples by Dr. Philip M. Cook (U.S. EPA. Duluth, MN).

^b Congener ratios are defined as pmol/g egg congener 1 divided by pmol/g egg congener 2.

NA: not available. Source: Zabel, *et al.*, 1995.

Figure 1. Structures of Dioxin-like Compounds (from Nevalainen and Kolehmainen, 1994).

The coefficients for dioxin-like activity for chlorine substituents at different positions in the dibenzo-*p*-dioxin (PCDD), dibenzofuran (PCDF), biphenyl (PCB) and diphenyl ether rings are shown. A positive value indicates positive activity and a negative (-) sign, negative activity.

Table 3. TEQs in pg/g lipid for all individuals 20+ years of age in the US population

TEQ	Percentiles	TEQ (1997)	95% CI	TEQ (2005)	95% CI	n
Total	90th	54.6	47.3–61.8	41.0	35.8–47.1	1194
	95th	68.9	62.9–80.8	56.1	47.6–65.4	1194
PCDD	90th	25.7	20.6–30.3	25.8	20.8–30.4	1194
	95th	34.8	28.7–43.3	34.8	28.7–43.4	1194
PCDF	90th	9.8	8.5–11.4	7.1	6.1–8.3	1194
	95th	12.3	11.0–14.4	8.9	7.7–10.2	1194
Coplanar PCB	90th	7.2	6.4–8.4	8.0	7.2–9.2	1194
	95th	11.0	9.7–12.0	11.9	10.8–12.9	1194
Mono-ortho PCB	90th	14.1	12.0–15.6	2.0	1.7–2.3	1194
	95th	18.0	15.1–20.4	2.6	2.3–3.0	1194

Source: Patterson *et al.*, 2008.

Table 4. Comparison of combustion emissions TEQ calculation by I-TEF, WHO-97, and WHO-05

Dioxin samples:				San Bernardino 11/27/88 (a)				Marion Co. incinerator (b)			
		WHO-97	WHO-05			WHO-97	WHO-05			WHO-97	WHO-05
Congener	I-TEF	TEF	TEF	pg/m ³	I-TEQ	TEQ	TEQ	pg/m ³	I-TEQ	TEQ	TEQ
2,3,7,8-TCDD	1	1	1	0.0106	0.0106	0.0106	0.0106	81	81	81	81
1,2,3,7,8-PeCDD	0.5	1	1	0.048	0.024	0.048	0.048	9	4.5	9	9
1,2,3,4,7,8-HxCDD	0.1	0.1	0.1	0.076	0.0076	0.0076	0.0076	7	0.7	0.7	0.7
1,2,3,6,7,8-HxCDD	0.1	0.1	0.1	0.063	0.0063	0.0063	0.0063	8	0.8	0.8	0.8
1,2,3,7,8,9-HxCDD	0.1	0.1	0.1	0.066	0.0066	0.0066	0.0066	8	0.8	0.8	0.8
1,2,3,4,6,7,8-HpCDD	0.01	0.01	0.01	0.429	0.00429	0.00429	0.00429	138	1.38	1.38	1.38
OCDD	0.001	0.0001	0.0003	0.93	0.00093	0.000093	0.000279	184	0.184	0.0184	0.0552
2,3,7,8-TCDF	0.1	0.1	0.1	0.024	0.0024	0.0024	0.0024	168	16.8	16.8	16.8
1,2,3,7,8-PeCDF	0.05	0.05	0.03	0.035	0.00175	0.00175	0.00105	10	0.5	0.5	0.3
2,3,4,7,8-PeCDF	0.5	0.5	0.3	0.035	0.0175	0.0175	0.0105	15	7.5	7.5	4.5
1,2,3,4,7,8-HxCDF	0.1	0.1	0.1	0.081	0.0081	0.0081	0.0081	4	0.4	0.4	0.4
1,2,3,6,7,8-HxCDF	0.1	0.1	0.1	0.035	0.0035	0.0035	0.0035	4	0.4	0.4	0.4
2,3,4,6,7,8-HxCDF	0.1	0.1	0.1	0.033	0.0033	0.0033	0.0033	5	0.5	0.5	0.5
1,2,3,7,8,9-HxCDF	0.1	0.1	0.1	0.033	0.0033	0.0033	0.0033	5	0.5	0.5	0.5
1,2,3,4,6,7,8-HpCDF	0.01	0.01	0.01	0.404	0.00404	0.00404	0.00404	7	0.07	0.07	0.07
1,2,3,4,7,8,9-HpCDF	0.01	0.01	0.01	0.086	0.00086	0.00086	0.00086	10	0.1	0.1	0.1
OCDF	0.001	0.0001	0.0003	0.252	0.000252	0.0000252	0.0000756	36	0.036	0.0036	0.0108
Total TEQ (pg TCDD equiv/m ³)					0.105322	0.1282582	0.120795		116.17	120.472	117.316

(a) Ambient dioxin data. From: letter from ENSR to Mr. R. Propper, CARB, 5/16/1989. In: CARB, 1990. Many of the entered levels are based on detection limit. (b) Flue gas emissions, municipal waste/power generation facility, Marion Co., OR. From: Dioxin Emissions from Resource Recovery Facilities: a review of the existing data base. CAPCOA/ARB/USEPA, 1989.

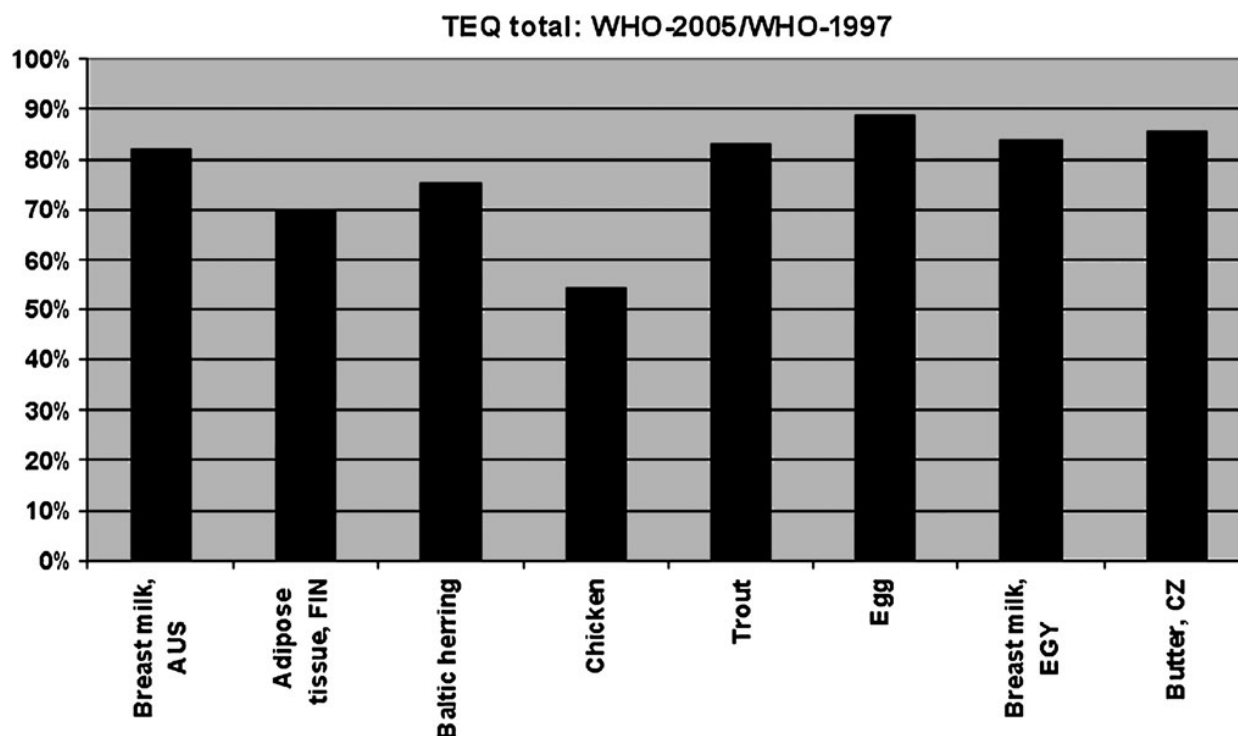
Neither of these sources reported measuring any levels of PCBs. Any such additional contaminants, if present, would add to the total TEQ observed by the WHO-97 TEF or WHO-05 TEF method, but not by the I-TEF method.

Table 5. Comparison of striped bass TEQ calculation by I-TEF, WHO-97 TEF, and WHO-05 TEF

Dioxin/PCB Congener	I-TEF	WHO-97 TEF	WHO-05 TEF	pg/g	I-TEQ	WHO-97 TEQ	WHO-05 TEQ
2,3,7,8-TCDD	1	1	1	0.13	0.13	0.13	0.13
1,2,3,7,8-PeCDD	0.5	1	1	0.2	0.1	0.2	0.2
1,2,3,4,7,8-HxCDD	0.1	0.1	0.1	0.24	0.024	0.024	0.024
1,2,3,6,7,8-HxCDD	0.1	0.1	0.1	0.16	0.016	0.016	0.016
1,2,3,7,8,9-HxCDD	0.1	0.1	0.1	0.21	0.021	0.021	0.021
1,2,3,4,6,7,8-HpCDD	0.01	0.01	0.01	0.17	0.0017	0.0017	0.0017
OCDD	0.001	0.0001	0.0003	0.3	0.0003	0.00003	0.00009
2,3,7,8-TCDF	0.1	0.1	0.1	0.74	0.074	0.074	0.074
1,2,3,7,8-PeCDF	0.05	0.05	0.03	0.14	0.007	0.007	0.0042
2,3,4,7,8-PeCDF	0.5	0.5	0.3	0.31	0.155	0.155	0.093
1,2,3,4,7,8-HxCDF	0.1	0.1	0.1	0.14	0.014	0.014	0.014
1,2,3,6,7,8-HxCDF	0.1	0.1	0.1	0.14	0.014	0.014	0.014
2,3,4,6,7,8-HxCDF	0.1	0.1	0.1	0.17	0.017	0.017	0.017
1,2,3,7,8,9-HxCDF	0.1	0.1	0.1	0.18	0.018	0.018	0.018
1,2,3,4,6,7,8-HpCDF	0.01	0.01	0.01	0.28	0.0028	0.0028	0.0028
1,2,3,4,7,8,9-HpCDF	0.01	0.01	0.01	0.2	0.002	0.002	0.002
OCDF	0.001	0.0001	0.0003	0.29	0.00029	0.000029	0.000087
PCB 77	0	0.0001	0.0001	64.5	0	0.00645	0.00645
PCB 81	0	0.0001	0.0003	nr			
PCB 105	0	0.0001	0.00003	1000	0	0.1	0.03
PCB 114	0	0.0005	0.00003	nr			
PCB 118	0	0.0001	0.00003	3000	0	0.3	0.09
PCB 123	0	0.0001	0.00003	nr			
PCB 126	0	0.1	0.1	23.3	0	2.33	2.33
PCB 156	0	0.0005	0.00003	0	0	0	0
PCB 157	0	0.0005	0.00003	0	0	0	0
PCB 167	0	0.00001	0.00003	nr			
PCB 169	0	0.01	0.03	2	0	0.02	0.06
PCB 189	0	0.0001	0.00003	0	0	0	0
TEQ Dioxins (pg TCDD equiv/g)					0.59709	0.696559	0.638327
TEQ PCBs (pg TCDD equiv/g)					0	2.75645	2.51
Total TEQ (pg TCDD equiv/g)					0.59709	3.453009	3.148327

Notes: Adapted from: SFBRWQCB, 1995. Tissue sample from a striped bass caught in San Francisco Bay. I-TEF values were introduced for PCBs in 1994 but these were not adopted by the California Air Toxics program. nr = not reported.

Figure 2. Percentage of reduction in total TEQ levels calculated for the same biotic samples when WHO-05 TEFs rather than WHO-97 TEFs are used



For each biotic sample shown, the height of the bar is the percentage that the total TEQ level determined using WHO-05 TEFs is of the total TEQ level determined using WHO-97 TEFs.

Source: van den Berg *et al.*, 2006.

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